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An ecological survey of the invertebrate community at the epigean/hypogean interface

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Abstract

We studied the ecological continuum between caves and the associated network of fissures – *Milieu Souterrain Superficiel* (MSS) – in an hypogean site in the Graian Alps, Italy. Over one year, we surveyed the faunal assemblages by means of pitfall traps placed in the caves and specific subterranean sampling devices (SSD) buried in the MSS. We used generalized linear mixed models (GLMMs) and generalized additive mixed models (GAMMs) to compare the spatial and temporal dynamics of the subterranean invertebrates inhabiting the two environments. As expected, arthropod communities occurring near the surface were characterized by minor level of subterranean adaptations, and conversely, subterranean species were more abundant and diversified at higher depths, both in the caves and in the MSS. Diversity and abundance of external elements in the superficial layers were found to be highly seasonal dependent, with minor values in winter compared to the other seasons. We provided information about the faunal assemblages dwelling in the two hypogean compartments, and we characterized the microclimatic conditions therein. We discussed the existence of an ecological gradient of specialization extending from the surface to the deep hypogean layers, which can be interpreted in light of the microclimatic changes occurring at increasing depths and the parallel decrease in available organic matter.

Keywords

Mesovoid Shallow Substratum, Cave fauna, Superficial Subterranean Habitats, Subterranean biology, Subterranean Sampling Device, Ecological gradient, Troglobionts

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Introduction

According to the modern view of subterranean biology, subterranean organisms do not exclusively inhabit underground vacuums of wide dimensions (i.e. caves), but also naturally occupy the network of fissures the size of which is not commensurable to the human scale (Racovitza 1907, Jeannel 1926, 1942, 1943, Juberthie et al. 1980a, 1980b, 1981, Uéno 1987). Culver and Pipan (2009a, 2014) recently categorised the most superficial subterranean habitats colonized by a strictly subterranean fauna as "Shallow Subterranean Habitats (SSHs)". Among the variety of SSHs listed by Culver and Pipan, the "Milieu Souterrain Superficiel (MSS)" is possibly one of the most inventively studied (Mammola et al. 2016). As a general definition, the MSS consists of a labyrinth of air-filled voids within rocky fragments that have accumulated for various morphogenetic reason on the bedrock, harbouring organisms showing adaptation to the subterranean conditions. In a hypothetical multilayer-structure, the MSS is generally found between the edaphic area – soil and rhizosphere – and the deep hypogean domain resulting, as a whole, in a "gradient from soil to cave" (Gers 1998). The climatic isolation of the MSS from the surface is usually achieved when the rocky layers are progressively covered by evolving soils with edaphic horizons (Giachino and Vailati 2010, Pipan et al. 2011), but other insulation mechanisms may be involved (Mammola et al. 2016). As a general rule, the external climatic variations are buffered in the MSS according to increasing depth (Nitzu et al. 2010, 2014), i.e. approaching a cave-like climate at higher distances from the surface (Badino 2010, Mammola et al. 2016).

Since MSS is not accessible to men unless by indirect means (see, e.g., López and Oromí 2010), biological studies focusing on the subterranean fauna are usually set in caves or in more accessible SSHs, such as lava tubes (e.g., Howarth 1972, Aschmole and Ashmole 1997, Arnedo et al. 2007). As a matter of fact, the great majority of the published papers investigating the MSS dealt with single model taxa, and were usually strictly taxonomical (Mammola et al. 2016). The focus of the MSS studies was rarely set on the whole community and/or on ecological processes (but see, e.g., Gers 1998, Nitzu et al. 2010, Pipan et al. 2011, Rendoš et al. 2012, Ortuño et al. 2013, 2014, Langourov et al. 2014, Jimenéz-Valverde et al. 2015). As an example, all the studies conducted so far in Italy focused on taxonomic descriptions of new species, sampled in the MSS by means of buried pitfall traps or sight-collected after excavation (e.g. Monguzzi 1982, 2011, Casale and Rondolini 1983, Casale and Giachino 1988, Vailati 1988, Latella and Rampini 1994, Monguzzi and Regalin 2001, Giachino and Vailati 2008, Magrini et al. 2012, Monzini 2013).

We conducted a one-year ecological study in an alpine hypogean site, aiming at investigating simultaneously the cave environment and the surrounding MSS. Our aims were to 1) compare the faunal assemblages characteristic of the two subterranean compartments; 2) investigate whether a temporal (seasonal) and/or a spatial gradient of specialization exists in the MSS – i.e. higher richness and abundance of specialized elements at increasing depth; 3) investigate whether the same gradients exist in the cave – i.e. variation in richness and abundance related to season and/or to vertical distance from the surface (subjacency) or to distance from the cave entrance.

Materials and methods

Study area

The study was set in the Pugnetto hypogean complex, in the nearby to the hamlet of Pugnetto, municipality of Mezzenile, Lanzo Valley, Graian Alps, Piedmont (NW-Italy). The site is protected under the European Habitat Directive 43/92 (S.C.I. IT 1110048) and hosts five natural caves classified as "Caves not open to the public" (H 8310), namely the Borna di Pugnetto (cadastre number Pi 1501, entrance at N45°16'19", E5°02'26"; altitude 820 m a.s.l.), the Tana del Lupo (Pi 1502, N45°16'19", E5°02'22"; 813 m a.s.l.), the Creusa d'le Tampe (Pi 1503, N45°16'12", E5°02'33"; 870 m a.s.l.), the Tana della Volpe (Pi 1504, N45°16'13", E5°02'34", 885 m a.s.l.) and the Cavernetta (N45°16'17", E5°02'36", 895 m a.s.l.) which still lacks a cadastre number but was mentioned in the original description of the hypogean site by Muratore (1946).

Our study was conducted in the Borna di Pugnetto (hereinafter Borna) and in the Creusa d'le Tampe (hereinafter Creusa) caves (Figs 1a, b, d; 2), as well as in the surrounding MSS. The Borna has a planimetric development of 765 m. It consists of a main gallery of 300 m directed north-south, which splits at the end into two main branches – "Ramo della Madonna" and "Ramo della Fontana". The Creusa has a planimetric development of 47 m. It consists of a single tunnel, which leads to the final chamber after a narrow passage. The area surrounding the caves is characterized by a well-developed colluvial MSS made of fragmented rocks (calcschist, marble and phyllite) which form a network of habitat spaces with sizes between 0.1 and 10–20 cm, covered by 30–40 cm of leaf litter and soil (Fig. 1c, e). Surface conditions are characterized by a close and shaded *Luzulo-Fagetum* beech forest (Sindaco et al. 2009).

Traps and sampling design

We used twenty-four pitfall traps (diameter 9 cm, volume 40 ml) to collect invertebrates in caves. The pitfall traps were arranged in groups of three (hereinafter cave-triplets), at a distance of ~5 m to one another. Six cave-triplets (code: C1–C6) were placed in the Borna at 4, 30, 90, 150, 230 ("Ramo della Madonna") and 350 ("Ramo della Fontana") meters from the main entrance. Two cave-triplets (code: C7, C8) were placed in the Creusa at 5 and 25 meters from the main entrance (Fig. 2). We derived the subjacency (DEPTH; i.e., the direct vertical distance from the surface) of each cave triplet from the geological survey of Motta and Motta (2015). Specifically, we created three categories of subjacency (Fig. 2): 0–20 m (triplets C1, C5, C7, C8), 20–40 m (C2, C6), and 60–80 m (C3, C4) [see Mammola et al. (2015) for details].

For collecting invertebrates in the MSS, we utilized twenty-four Subterranean Sampling Devices (SSD; after López and Oromí 2010). Since the description of the MSS by Juberthie et al. (1980a, 1980b, 1981) and Uéno (1980), there has been considerable effort to construct and improve effective sampling techniques in this habitat

Figure 1. a Main entrance of the Borna di Pugnetto (photo credit: Alberto Chiarle and Mauro Paschetta, 2014) **b** Main entrance of the Creusa d'le Tampe (photo credit: Elena Piano 2013) **c** exposed soil/MSS profile in a fresh-cut along a slope in the vicinity of the Borna di Pugnetto (photo credit: Jacopo Orlandini, 2014) **d** the typical cave geo-morphology within the Borna di Pugnetto (photo credit: Alberto Chiarle and Mauro Paschetta, 2014) **e** detail of the MSS geo-morphological structure (photo credit: Jacopo Orlandini, 2014).

(reviewed in López and Oromí 2010, Domingo-Quero and Alonso-Zarangara 2010, Mammola et al. 2016, Růžička and Dolanský 2016). In this study, we constructed SSDs relying on the prototypes of Nitzu et al. (2010, 2014) and Deltshev et al. (2011). SSDs consisted of PVC pipe (diameter 12.5 cm) with a silicone cap closing the top. A straight row of 25 holes (diameter: ~1.5 cm) was pierced at a distance of 8 cm from the lower-end of each pipe (Fig. 3a). At the same height, in correspondence of the level of holes row, we placed a standard pitfall trap. The holes drilled along the surface of the PVC pipe allowed the fauna to access to the inside and eventually fall into the pitfall trap; at the same time, our SSD allowed to recover, empty, refill and replace the pitfall trap trough the closing cap, ensuring that the substrate was not mechanically excavated at each sampling session (Fig. 3f, g). In addition, we inserted a styrofoam cylinder within the PVC tube, filling completely the pipe and insulating the trap from the external meteorological fluctuations. A screw was placed just above the sampling holes, to prevent the styrofoam cylinder from sliding down (Fig. 3b).

Three adjacent SSDs of three different length (40, 60 and 80 cm) were buried vertically in the ground (hereinafter MSS-triplet; Fig. 3c, d, e). The 40 cm SSD intercepted the substrate at the interface soil/MSS, while the 60 and 80 cm SSDs inter-

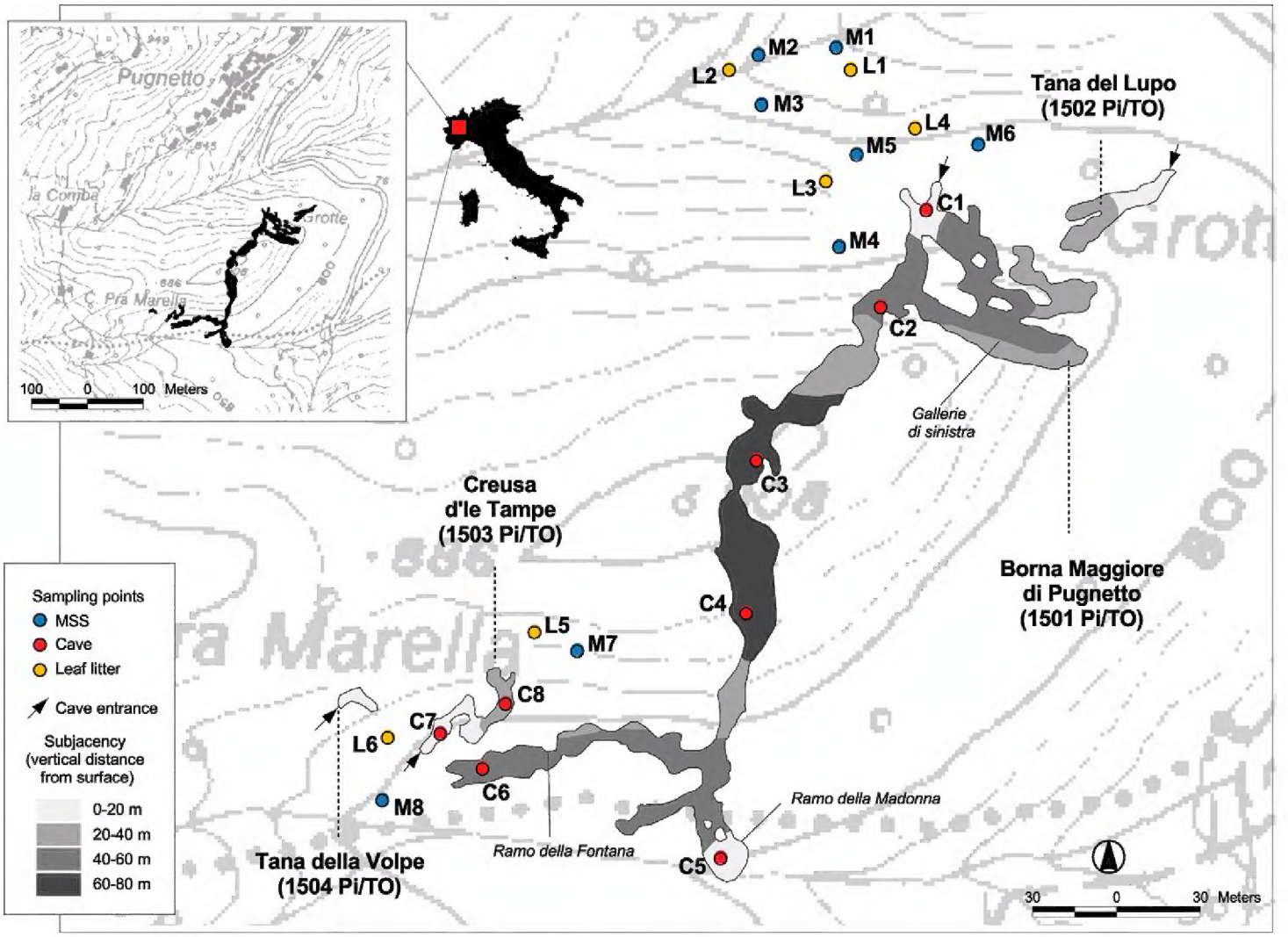

Figure 2. Map of the study area. The shape and the topographic position of the four caves (Borna Maggiore di Pugnetto, Tana del Lupo, Creusa d'le Tampe, Tana della Volpe) was obtained from the original planimetric drawings of Muratore (1946). The position of the sampling plots in caves ("cave triplets", C1–C8), in the MSS ("MSS triplets", M1–M8) and in the leaf litter ("epigean", L1–L6) are represented by coloured dots. The different sectors of the cave are coloured with different shades of grey representing the subjacency – i.e., vertical distance from the surface – according to Motta and Motta (2015).

cepted the MSS. Six MSS-triplets (18 SSDs; code: M1–M6) were set in the nearby of the Borna, and two MSS-triplets (6 SSDs; code: M7, M8) were placed in the nearby of the Creusa (Fig. 2). We installed the traps in the MSS following the advices reported in literature (Domingo-Quero and Alonso-Zarangara 2010, Giachino and Vailati 2010, Lopez and Oromì 2010, Mammola et al. 2016; see Fig. 3). Pitfall traps in caves and in the MSS were baited with chicken meat and filled with brine (supersaturated preserving solution of water and NaCl; Giachino and Vailati 2010).

Six pitfall traps (with brine, not baited) were also placed in leaf litter habitat (epigean; code: L1–L6), as a reference to discriminate correctly between the epigean fauna and the specialized fauna – see paragraph "Specimens sorting and ecological classification" for details.

Figure 2 shows the position of the traps in the area. We replaced the traps approximately once a month from June 2012 to June 2013. However, the access to the Borna is forbidden from 1st November to 31th March in order to protect the roosting bats inhabiting the cave. In order to reduce disturbance, in this period we accessed

Figure 3. a sampling holes (details) **b** Blocking screw **c** installation of an MSS-triplet **d** SSD of three different length **e** MSS-triplet buried in the ground **f, g** renewing the pitfall trap inside the SSD. Photo credits: Elena Piano.

the Borna only twice (December and March), resulting in ten total sampling sessions over the year. In order to evaluate the effect of the bait on the sampling probability, we replaced it every two sampling sessions, thus resulting in 5 sampling sessions with fresh and 5 with exhausted baits.

To characterize the subterranean microclimate, we placed one Hygrochron temperature and humidity datalogger in correspondence of each pitfall trap in the cave and at the lower-end of each SSD. Hygrochron were programmed to sample temperature (T) and relative humidity (RH) every three hours for the whole sampling period (accuracy of ± 0.5°C and ± 1%, respectively). We also derived the mean daily outside

temperature to the same periods from the nearest thermo-hygro-pluviometric weather station (Fua, Lanzo Torinese, Cod. 111; N45°17'23", E7°29'38"; 550 m a.s.l.). The temperature values recorded by the weather station were corrected with the standard environmental lapse rate – the change of temperature with altitude for the stationary atmosphere. In all analyses relating the abundance and species richness of external and adapted elements with the explanatory parameters (see later sections), we used the pseudo-replicates of each trap as basic sample units.

Specimens sorting and ecological classification

Trapped individual were sorted, identified and classified either as epigean (category: "external") or subterranean elements (category: "adapted"). According to our expertise (MI and SM: Araneae; PMG: Coleoptera and some other orders of Insecta) and the availability of specialists for additional *taxa* (see Acknowledgments), identification of the species levels was possible for the orders Araneae, Opiliones, Pseudoscorpiones, Chilopoda, Isopoda (one species), and for most orders of insects (especially Coleoptera; see Appendix 1).

In subterranean biology, species are often classified into ecological categories (e.g. *trogloxenes*, *troglophiles*, *troglobionts*) according to their preferred habitat and general association with the subterranean domain (Sket 2008, Trajano and Carvalho 2017). However, most ecological classifications, such as the Schiner-Racovitza system, are deemed to oversimplify real cases given that boundaries between the categories are often vague and difficult to attribute (Martìn et al. 2001, Giachino and Vailati 2016). Alternatively, in this work, to categorise our samples we followed the general idea based on a source-sink population model recently proposed by Trajano (2012). By examining the prevalence of individuals either in the leaf litter traps or in the traps installed in the subterranean habitats, we were able to classify species as "external" (species likely having source populations in the epigean environment) or "adapted" (species likely having source populations in the hypogean environment). Following this criterion, we calculated the abundance and species richness of external and subterranean elements (Next, Rext, Nad, Rad, respectively) for each trap in each sampling survey. Species represented by less than three individuals were not classified, and thus excluded from the analysis, unless if we possessed a solid literature background testifying the species ecological requirements – e.g., Lana (2001) for a few subterranean species in Piedmont and Mammola et al. (2017) for most spider species.

Statistical analysis

We performed regression-type analysis following the general advices of Zuur and Ieno (2016). All analysis were conducted in R (R development core team 2015). In order to test statistically our working hypothesis, we computed three distinct sets of regression models. Firstly, we compared the cave and the MSS habitats in term of species richness

and abundance of external and adapted organisms (aim 1). Subsequently, we analysed the spatial and temporal gradients in the MSS and cave environments separately, aiming at investigate the relevant abiotic factors driving richness and abundance in these two hypogean compartments (aims 2 and 3).

The first set of models was computed through mixed-design analysis of variance with Poisson distributed data (i.e. generalized linear mixed models, GLMMs; equation 1), whereas for the second (equation 2) and third (equation 3) sets, we primarily relied on Poisson generalized additive mixed models (GAMMs). The mixed part of both GLMMs and GAMMs was introduced in order to account for multiple observations from the same triplet over time, by specifying the triplet as random factor. The latter variable was included as random factor in order to account for the variation it introduced in our samples – and thus to correctly estimate the regression coefficients, – rather than to test for its direct effect on the dependent variables.

Prior to model fitting, we explored the three datasets following the standard protocol for data exploration proposed by Zuur et al. (2010). According to Zuur et al. (2009, 2010), the inclusion of outliers and highly correlated predictors in the regression analysis leads to misleading results – type I and II statistical errors. We thus used Cleveland's dotplots to assess the presence of outliers in dependent and independent variables and we investigated multi-collinearity among covariates. For each basic sample unit and for each set of models, we chose the abundance of external elements (Next), the species richness of external elements (Rext) and the abundance of adapted elements (Nad) as dependent variables. Such variables were selected aiming at investigate the gradient of specialization trough the hypogean environment (aims 2 and 3). Instead, considering the generally low species richness of adapted elements per trap (Rad), we were forced to exclude this latter variable from our analysis. For both abundance (Next, Nad) and richness (Rext) count data we assumed a Poisson distribution, but we tested for over-dispersion after model fitting and we switched to a negative binomial distribution when the over-dispersion parameter was higher than 1.2.

Comparing MSS and cave communities (aim 1)

Species richness of external elements and abundance of adapted and external elements were analysed in relation to the explanatory two-levels factor HABITAT (levels: "Cave" and "MSS") using the *glmer* R command in the package 'lme4' (Bates et al. 2013).

The fixed structure of the model was:

(1) y ~ HABITAT

Where y is one of Next, Rext and Nad. The random part of the model allowed us to deal with repeated observations and measurements of the same triplet (temporal dependence) and the clumped distribution of the traps within the triplet (spatial dependence).

Spatial and temporal gradients in MSS (aim 2) and cave (3) communities

Species richness of external elements and abundance of adapted and external elements were analysed in relation to the explanatory variables using the *gamm* R command in the package 'mgcv' (Wood 2015). Generalized additive mixed model were used in order to account for possible non-linear trends of the sampling period (Serie_i; continuous variable). The optimum amount of smoothing was estimated trough generalized cross-validation (GCV). However, whenever the effect was linear and/or not significant, we dropped the smoothed term and fitted a new model including only parametric terms, thus adopting a linear approach (Poisson or negative binomial GLMM).

For both the cave and the MSS compartments, we also included the two-levels factor BAIT (level= "Fresh" and "Exhausted") as covariates and the two-levels factor SITE (levels= "Borna" and "Creusa"). The first variable was included to evaluate the effect of the ageing of the bait on our dependent variables. The second variable was introduced to take into account for possible local effects, since we pooled together records from two different caves and associated MSS.

For the analysis of the MSS compartment, in addition to the above mentioned variables we also included in the models the three-level categorical variable sampling depth (DEPTH; levels: "0.4m", "0.6m" and "0.8m"). For the cave habitat, in addition to the aforementioned variables, we also included the distance from the cave entrance (Dst_i; continuous variable) and the three-levels categorical variable subjacency (SUBJ; levels: "0–20m", "20–40m" and "60–80m"). We excluded from the regression analysis the microclimatic variables, given that due to malfunctioning, several dataloggers did not recorded reliable measurements of temperature and relative humidity during the sampling period (see results, further details in Mammola et al. 2015).

The (fixed) structures of the initial models were (aims 2 and 3, respectively):

(2) y ~ DEPTH + s(Serie_i) + BAIT + SITE
(3) y ~ Dst_i + SUBJ + s(Serie_i) + BAIT + SITE

Where y is one of Next, Rext and Nad, and s(Serie_i) indicate the smoothing term. The random part of the model is equal to the previous model (1). For the 2nd and 3rd models, we adopted a statistical hypothesis testing framework, whereby model reduction was carried out on the full model by sequentially deleting non-significant terms and potential interactions according to AIC values (Zuur et al. 2009), until a minimum adequate model of significant fixed effects remained (i.e. best model supported by observations). For all models, p-values for parametric term were based on z-tests; t-test in the case of GAMMs and GLMM with negative binomial distribution. P-values for smoother terms represent approximate significance based on F statistic. Model validation was carried out on the final models, following the approach of Zuur et al. (2009).

Results

General considerations

Approximately 15,700 Arthropoda (20 Orders), 117 Mollusca (order Pulmonata), 868 Crustacea (order Isopoda), and 14 Anellida (*Lumbricus* sp.) were collected. We report the complete list of the taxa and number of specimen collected in each habitat in Appendix 1. Diptera were the most represented, with 7,469 individuals collected (adults and larvae), followed by Coleoptera (3,543 individuals; adults and larvae), Collembola (1,755 individuals), Hymenoptera (1,046 individuals, mostly Formicidae) and Acari (631 individuals). MSS showed higher values of order diversity compared to caves, in which, abundance of specimens was higher.

Cave and MSS microclimate

Due to malfunction, several dataloggers did not sample either temperature or relative humidity during the sampling period. Specifically, nine out of eighteen in the Borna, two out of six in the Creusa and ten out of twenty-four in the MSS. Due to this significant loss of data, it was not possible to include climatic data in the regression analyses.

Relative humidity in the caves proved to be almost constantly close to saturation, with mean monthly values ranging from 85 to 100 %. However, in the vicinity of the entrance the relative humidity dropped down to 70–75 % in winter. In the MSS the mean monthly relative humidity was always above 90 %. With regard to temperature, changes and max–min ranges were attenuated with increasing distance from the entrance and delayed in respect to the outside values. The mean annual temperature values deep inside the two caves was comparable (Tmean ± SD: Borna = 9.0 ± 0.4 °C; Creusa = 8.9 ± 0.8 °C) and presented little variations over the year. In the outermost sections, temperature was nearly stable in summer, spring and autumn, with a max–min range around 4.5 °C in the vicinity of the entrance for both caves. However, the microclimate at the entrance zone drastically changed during winter, when we observed a drop in the mean temperature values (mean values always below 6 °C). The coldest temperature values were recorded in December and January (Tmin: Borna = –2.0 °C and Creusa = –0.9 °C).

The range of temperature variation, both daily and monthly, was lower in the MSS in respect to surface (Fig. 4). In the MSS the thermal variability at the surface seemed to moderately affect the microclimatic conditions of MSS, resulting in seasonal variations in temperature at all sampled depths. The maximum temperature in the MSS did not exceed 19 °C even though air temperatures reached as high as 32.3 °C (June). Minimum temperature values rarely fell below 3 °C, with occasional drops below 0 °C (lower Tmin recorded in the MSS in December: –2.0 °C).

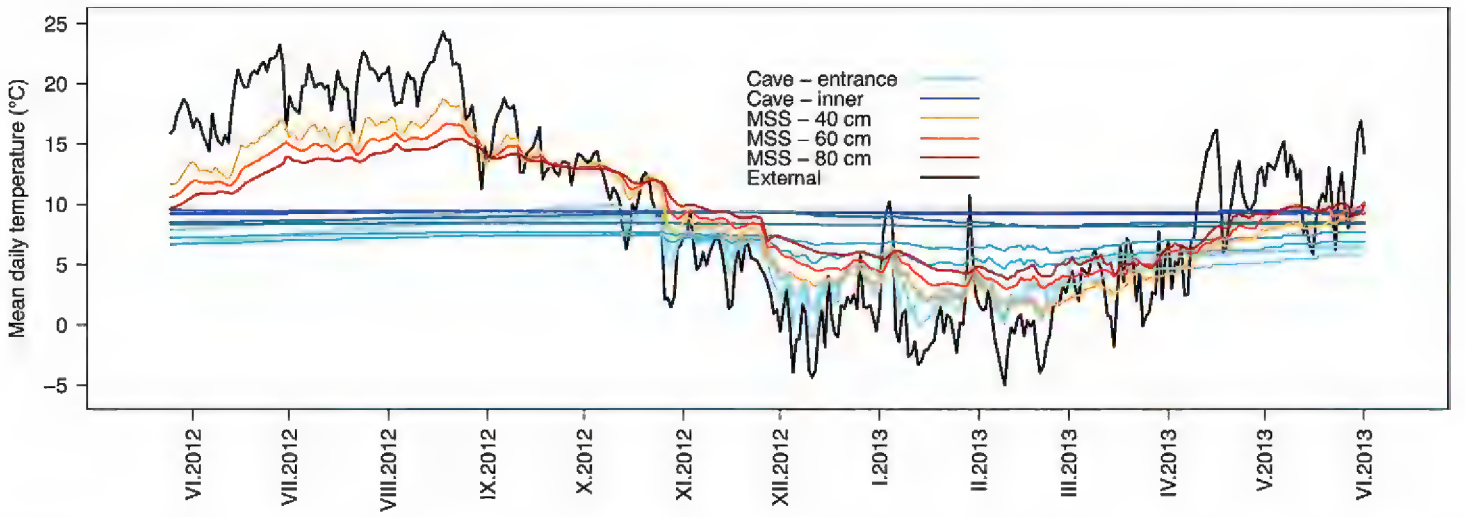

Figure 4. Annual trends of temperatures in the Pugnetto hypogean complex. The shade of blues indicate the relative position of the dataloggers at each cave-triplet, from the outermost (lighter blues) to the innermost sections (darker blues). Records from only one MSS-triplet are shown.

Cave and MSS comparison and faunal dynamics

Initial data exploration revealed the presence of a few outlying values (mostly due to higher prevalence of Diptera and Hymenoptera in certain traps), which were removed from the dataset. Both abundance and species richness of external elements were lower in caves in respect to MSS (Next: $t = -4.37$, $p < 0.001$; Rext: $z = -4.93$, $p < 0.001$). Conversely, the abundance of subterranean elements was higher in cave (Nad: $t = 5.39$, $p < 0.001$; Table 1).

The best model structures resulting from model selection concerning the analysis of MSS and caves (equations 2 and 3) are reported in Table 1. The analysis of the MSS data revealed contrasting patterns of species richness and abundance of external and subterranean elements according to sampling depth. The abundance and species richness of external elements was lower at –0.80 m (test relative to "0.40 m"; Next: $t = -3.217$, $p = 0.001$; Rext: $t = -3.283$, $p = 0.001$), whereas no significant effect was detected in respect to –0.60 m (Fig. 5a, b). The abundance of specialized elements was higher at –0.60 m (test relative to "0.40m"; Nad: $z = 2.481$, $p = 0.013$) and –0.80 m (test relative to "0.40m"; Nad: $z = 4.333$, $p < 0.001$; Fig. 5c). The ageing of the bait negatively influenced the abundance and richness of external elements, with higher values with fresh bait (test relative to "Exhausted"; Next: $t = 4.489$, $p < 0.001$; Rext: $t = 3.005$, $p = 0.002$). No effect was detected in respect to the abundance of adapted elements (Nad: $z = 4.333$, $p = 0.507$).

Abundance and richness of external elements showed a significant non-linear U-shaped trend in respect to the sampling series (Next: $F = 9.02$, $p < 0.001$; Rext: $F = 10.68$, $p < 0.001$), with higher values in summer and early autumn, followed by a drastic decline in winter and an uprising in spring (Fig. 6 a, b). There was no significant relationship between the sampling series and the abundance of adapted elements.

Concerning the cave habitat, we detected an higher abundance of external and adapted elements in the Creusa cave (test relative to "Borna"; Next: $t = 3.693$, $p < 0.001$;

Table 1. Estimated regression parameters and approximate significance of smooth terms according to GLMMs and GAMMs, respectively, obtained from the 3 sets of models and the 3 dependent variables considered (Next, Rext, Nad, see text). The final model structures resulting from model selection are reported (only fixed terms are shown).

	y	Final model	Model	Distribution	Variables	Parametric coefficients:			Smooth terms		
						Estimate (α or β)	SE	p	edf	F	p
MSS vs CAVE (Equation 1)	Next	~ HABITAT	GLMM	Negative binomial	Intercept (α)	0.9722	0.4490	–	–	–	–
					HABITAT (CAVE)	-2.9168	0.6669	<0.001 ***	–	–	–
	Rext	~ HABITAT	GLMM	Poisson	Intercept (α)	0.1418	0.2975	–	–	–	–
					HABITAT (CAVE)	-2.2727	0.4613	<0.001 ***	–	–	–
	Nad	~ HABITAT	GLMM	Negative binomial	Intercept (α)	-1.4751	0.4491	–	–	–	–
					HABITAT (CAVE)	3.2843	0.6087	<0.001 ***	–	–	–
MSS (Equation 2)	Next	~ BAIT + DEPTH + s(Serie_i)	GAMM	Poisson	Intercept (α)	0.5339	0.2475	–	–	–	–
					BAIT (Fresh)	0.9040	0.2014	<0.001 ***	–	–	–
					DEPTH (0.6m)	-0.1260	0.1939	0.516	–	–	–
					DEPTH (0.8m)	-0.7835	0.2436	0.001 **	–	–	–
					s(Serie_i)	–	–	–	3.321	9.02	<0.001 ***
	Rext	~ BAIT + DEPTH + s(Serie_i)	GAMM	Poisson	Intercept (α)	-0.440	0.1813	–	–	–	–
					BAIT (Fresh)	0.4196	0.1396	0.002 **	–	–	–
					DEPTH (0.6m)	0.0497	0.1410	0.724	–	–	–
					DEPTH (0.8m)	-0.5601	0.1706	0.001 **	–	–	–
					s(Serie_i)	–	–	–	4.478	10.68	<0.001 ***
	Nad	~ BAIT + DEPTH	GLMM	Poisson	Intercept (α)	-3.4961	0.6420	–	–	–	–
					BAIT (Fresh)	1.3337	0.2422	<0.001 ***	–	–	–
					DEPTH (0.6m)	0.8145	0.3283	0.013 *	–	–	–
					DEPTH (0.8m)	1.3131	0.3030	<0.001 ***	–	–	–

	y	Final model	Model	Distribution	Variables	Parametric coefficients:			Smooth terms		
						Estimate (α or β)	SE	p	edf	F	p
CAVE (Equation 3)	Next	~ SITE + s(Serie_i) x SUBJ	GAMM	Poisson	Intercept (α)	-2.7564	0.5078	–	–	–	–
					SITE (Creusa)	2.9922	0.8183	<0.001 ***	–	–	–
					s(Serie_i) x SUBj (0–20m)	–	–	–	5383	5.544	<0.001 ***
					s(Serie_i) x SUBj (20–40m)	–	–	–	1.000	0.193	0.661
					s(Serie_i) x SUBj (60–80m)	–	–	–	1.000	0.194	0.660
	Rext	~ SUBJ + Dst_i	GLMM	Poisson	Intercept (α)	–0.7185	0.2413	–	–	–	–
					SUBj (20–40m)	–1.0541	0.6363	0.097	–	–	–
					SUBJ (60–80m)	–2.5317	1.1017	0.021 *	–	–	–
					Dst_i	–0.0077	0.0029	0.010 *	–	–	–
	Nad	~ SITE + BAIT + SUBJ + Dst_i	GLMM	Negative binomial	Intercept (α)	1.3087	0.2343	–	–	–	–
					SITE (Creusa)	0.8874	0.2993	0.003 **	–	–	–
					SUBj (20–40m)	1.3205	0.2918	<0.001 ***	–	–	–
					SUBJ (60–80m)	0.6406	0.2853	0.024 *	–	–	–
					Dst_i	–0.0017	0.0009	0.072	–	–	–

Notes: BAIT = ageing of the bait (Categorical variable; levels: "Exhausted" and "Fresh"); Dst_i = distance from the cave entrance (Continuous variable); DEPTH = MSS sampling depth (Categorical variable; levels: "0.4m", "0.6m" and "0.8m"); HABITAT = habitat type (Categorical variable; levels: "Cave" and "MSS"); Serie_i = sampling series (Continuous variable); SITE = sampling site (Categorical variable; levels: "Borna", "Creusa"); SUBJ = subjacency (Categorical variable; levels: "0–20m", "20–40m", "60–80m"). The notation "s(Variable_i)" indicate continuous variables treated as smoothers. "x" denote statistical interaction. Significance codes: < 0.001 ***; < 0.005 **; < 0.05 *.

Nad: $t = 2.965$, $p = 0.003$), whereas the richness of external elements was not significantly influenced by this parameter (Fig. 5d, f). We detected a significant interaction between the sampling series and subjacency, with non-linear effects of the series in respect the abundance of external elements at 0–20m subjacency (Next at "0–20m": $F = 5.544$, $p < 0.001$). In the proximity of the surface, higher abundances were predicted during summer, autumn and spring, while a drastic decline was observed in winter (Fig. 6c). There was no significant interaction between the sampling serie and the other subjacency levels.

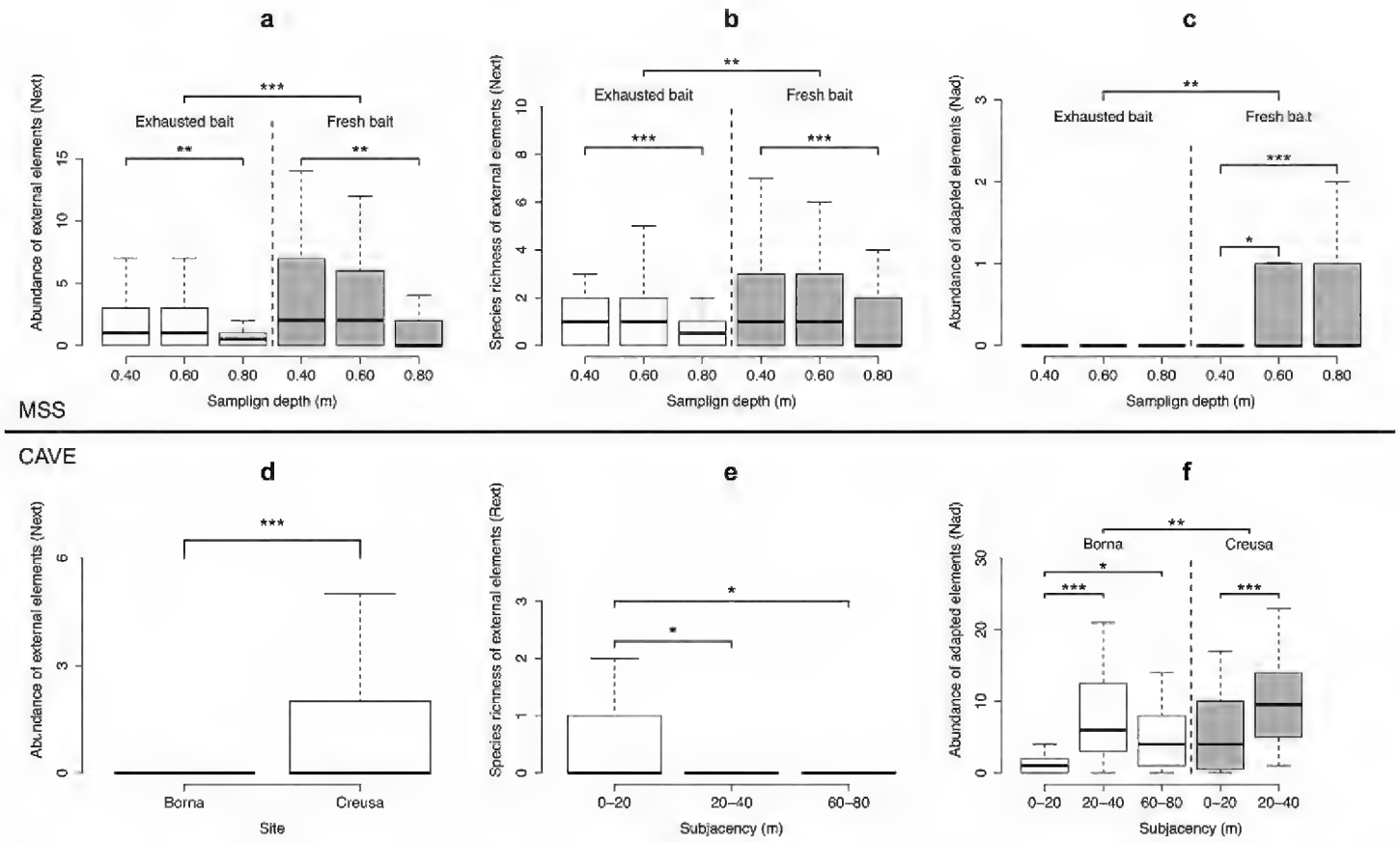

Figure 5. Boxplots showing the results of the regression analysis of the MSS (**a–c**) and the cave (**d–f**) data. Outlying values are not shown. Significance codes: < 0.001 ***; < 0.005 **; < 0.05 *.

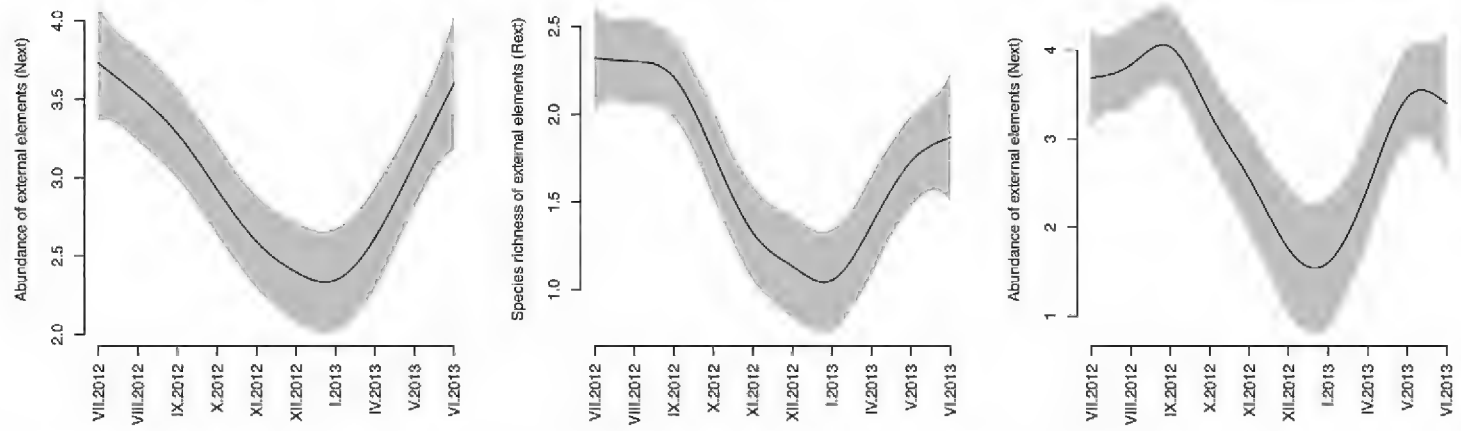

Figure 6. Predicted values (black line) and 95% confidence intervals (grey surface) of the effect of the sampling series (Serie_i) on the abundance of external elements in the MSS (**a**), on the species richness of external elements in the MSS (**b**) and on the abundance of external elements in the cave at subjacency of 0–20m (**c**) derived from GAMM analyses. Only fixed effects are shown.

We observed a decrease in the richness of external elements at increasing distance from the cave entrance (Rext; $z = -2.575$, $p = 0.010$). Moreover, the richness of external elements was lower at 60–80m subjacency (test relative to "0–20 m"; Rext: z = –2.298, $p = 0.021$). No significant effect was detected in respect to the 20–40m subjacency (Fig. 5e). There was also a significant increase in the abundance of subterranean elements at higher subjacency in respect to 0–20m (Nad at "20–40m": $t = 4.525$, $p < 0.001$; Nad at "60–80m": $t = 2.245$, $p = 0.024$; Fig. 5f). There was no significant relationship between the distance from the entrance and the abundance of adapted elements.

Discussion

Despite subterranean animal communities being relatively simple, their precise characterization still represents an interesting topic in subterranean ecology. This is mostly because spatial boundaries and species composition of the communities are difficult to define, especially when considering MSS and its interconnections with the deep hypogean domain. Relying on the classical definitions (Racovitza 1907, Juberthie 2000, Culver and Pipan 2009b), Sendra et al. (2014) recently defined the subterranean domain as "*[...] a network of intercommunicated micro, meso and macro voids, filled or not with meteoric water, extending from the surface towards the subsurface, allowing the movement of fauna communities and nutrients mostly from the surface.*" Accordingly, the connections between the superficial and the deep subterranean compartments represent an ideal ecological continuum with undefined boundaries, with the MSS acting as an extension of the deep hypogean domain toward the surface.

In this contribution, we aimed to investigate this ecological continuum both spatially and temporally, by comparing the arthropod communities inhabiting caves and the adjacent MSS compartments (Appendix 2). As far as we are aware, aside from this contribution, the only work in which a direct comparison between caves and the MSS is taken into consideration was the seminal study of Gers (1998), set in southwestern France. To some extent, we used a similar approach, by sorting out our samples on the basis of the degree of adaptation to subterranean life. However, we introduced a fresh approach, as we were able to discriminate objectively between subterranean and epigean organisms, thanks to the baseline provided by a series of control pitfall traps placed in the leaf litter (see paragraph "Specimens sorting and ecological classification" in the Materials and methods).

Although several species sampled at the Pugnetto hypogean complex are unique for obvious biogeographical reasons, the composition of the animal community was in general terms quite similar to that reported from other MSS sites in the Canary Islands (Pipan et al. 2011), Carpathians (Nitzu et al. 2010, 2014, Rendoš et al. 2012), Spain (Gilgado et al. 2014, Ortuño et al. 2014 Jiménez-Valverde et al. 2015), France (Juberthie and Decu 2006), and Bulgaria (Langourov et al. 2014). An abundance of individuals belonging to the main taxonomical groups sampled in the MSS, also aligned literature data (Borges 1993, Rendoš et al. 2012, Ortuño et al. 2013). In particular, we mostly collected arthropods, molluscs and anellids. The most represented orders were Diptera, Acari, Collembola and Coleoptera. In the case of Coleoptera, the proportion of individuals per family collected were, however, inverted in respect of the works of Juberthie and Decu (1998) and Moldovan (2005), in which Carabidae were more abundant than Cholevidae. In our study more than 60% of the captured beetles were Cholevidae and only ≈ 20% were Carabidae.

We found that the abundance of specialized organisms was higher in the cave compartment, whilst we documented a higher diversity and abundance of epigean species in the MSS. The dominance of epigean species in the superficial layers of the MSS has been reported by several authors (Medina and Oromí 1990, Crouau-Roy et al. 1992, Borges 1993, Gers 1998, Růžička and Thaler 2002, Deltshev et al. 2011, Pipan et al.

2011, Nitzu et al. 2010, 2014, Rendoš et al. 2012, Barranco et al. 2013, Langourov et al. 2014, Ortuño et al. 2013, 2014, Jimenéz-Valverde et al. 2015, among others). This result suggests that our survey actually detected the superficial layers of the MSS, which are intimately connected with the epigean and the edaphic mediums, and thus more easily colonized by external elements (Mammola et al. 2016).

Concerning the cave habitat, we detected a higher diversity and abundance in the Creusa rather than in the Borna (Fig. 5d, f). The same pattern was previously documented by Capra and Conci (1951). The explanation for this result could be twofold. Firstly, the larger entrance of the Creusa – almost four time wider than the Borna; Fig. 1a,b – results in higher energy inputs (especially beech leaves) and a higher occurrence of epigean species (Fig. 5d), which may, in turn, indirectly support higher density and diversity of strictly subterranean species (Fig. 5f). Secondly, the Creusa is characterized by high values of relative humidity, an environmental factor playing a key role for the subterranean fauna (Mammola et al. 2015: 247, f. 7).

When analysing the cave and MSS habitats separately, we detected a gradient of subterranean specialization of the biological community in both compartments. In the cave, regression analyses suggests that there was a gradient of subterranean specialization of the biological community from the entrance zone toward the deepest sectors (see, e.g., Tobin et al. 2013) – or, more generally, from shallow toward deep subterranean spaces (Laška et al. 2011). The richness and abundance of epigean, less adapted species was indeed higher at lower depths in MSS and at lower subjacency in the caves. Conversely, subterranean species were more abundant and diversified at higher depth and subjacency. These results mirror that of Růžička and Klimeš (2005), Giachino and Vailati (2010), Deltshev et al. (2011) and Ortuño et al. (2013). The increase of climatic stability related to depth (Fig. 4; Nitzu et al. 2010, 2014, Rendoš et al. 2016b) and the parallel decrease in available organic matter (Gers 1998, Rendoš et al. 2016b) convincingly explains these patterns.

At the same time, we observed how the presence of external elements was seasonally dependent, their abundance being highly fluctuating during the year. We observed this trend both in the MSS (Fig. 6a, b) and in the cave at reduced subjacency (Fig. 6c). According to the results of the GAMM, the prevalence of external elements dropped significantly during winter. Seasonal dynamics in faunal assemblages were observed by previous authors focusing on MSS (e.g., Crouau-Roy et al. 1992, Nitzu et al. 2011, 2014, Rendoš et al. 2012, 2016a) and on caves (e.g., Tobin et al. 2013, Ferreira et al. 2015, Mammola et al. 2015, Bento et al. 2016, Lunghi et al. 2017). It is possible to argue that, over the year, at the blurry epigean/hypogean interface, there are complex exchanges involving fauna with different levels of subterranean adaptation (Prous et al. 2004, Moseley 2009, Novak et al. 2012), which introduce fundamental trophic resources in the subterranean habitat (Novak et al. 2013) but also cause greater fluctuations in species composition over the year. Ultimately, these results suggest how boundaries between these habitats are very undefined and may vary with the season. In contrast, we did not detect any seasonal variation in respect to the occurrence of

subterranean elements both in the cave and in the MSS – i.e., the smoothed terms were not significant and we opted for regression models.

As a side question, with this study we were able to test the effect of the quality of the bait – at least up to two months – on the probability of capturing invertebrates in the two subterranean environments. This was possible since we renewed the bait in the trap every two sampling sessions, so that at each sampling session the condition of the bait changed from fresh to exhausted. According to the regression analysis, we demonstrated that a fresh bait is more effective in capturing individuals in the MSS (Fig. 5a, b, c) (i.e. that the bait is more attractive within the first month), unlike the cave compartment, in which we did not detect any significant effect. A similar trend is also discussed by Mammola et al. (2015).

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Appendix 1

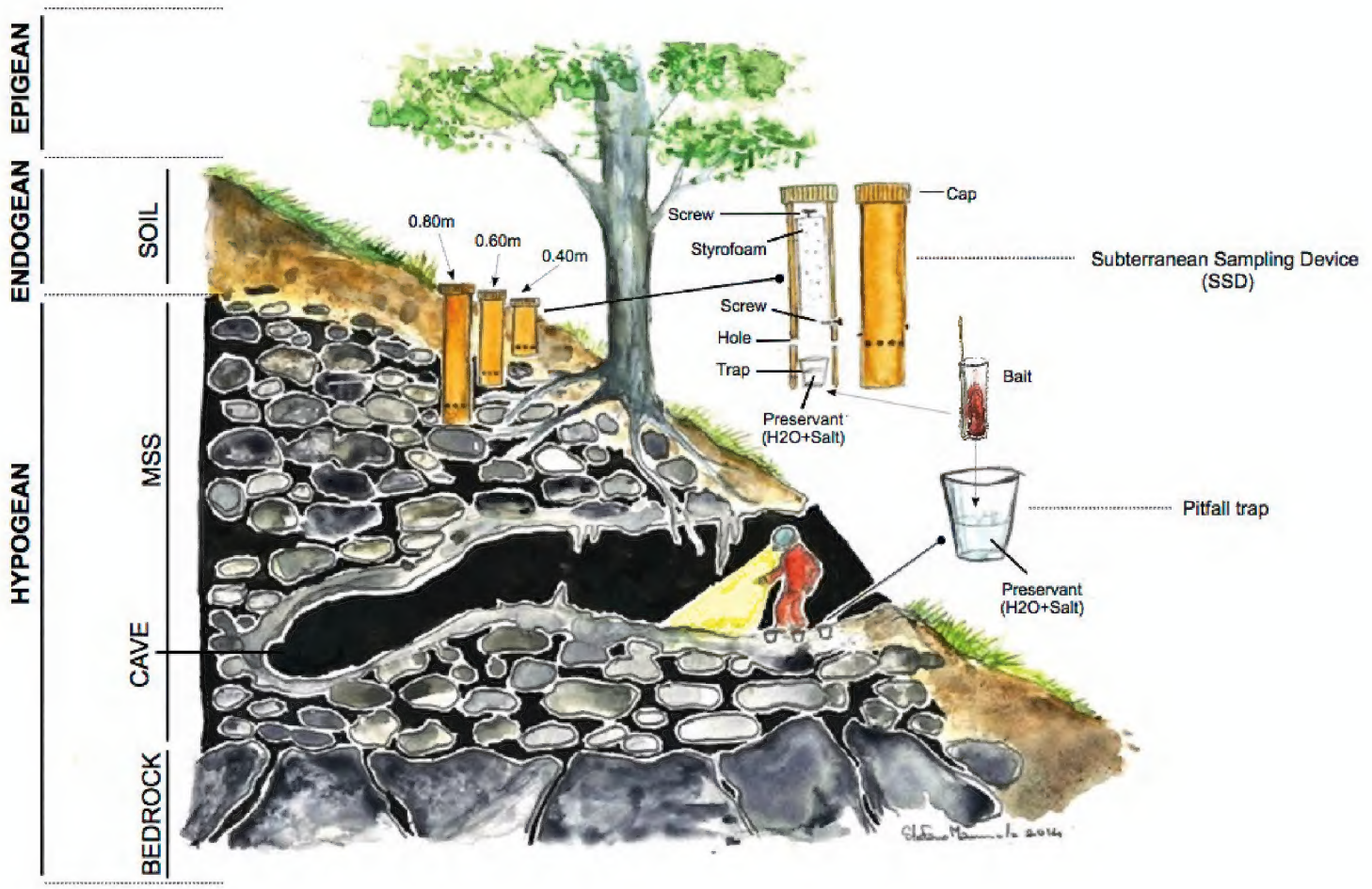
Taxonomic groups sampled in this study according to the habitat. The total number of sampled individuals in each habitat is reported. The column "adaptation" provides the attribution to the category "external" or "adapted". Classification was based on the criteria defined in the relative paragraph.

Class	Order	Family	Species	Adaptation	Litter	MSS	Cave
Arachnida	Acarina	Fam.	Morphospecies I	Adapted	87	24	137
Arachnida	Acarina	Fam.	Morphospecies II	External	75	–	–
Arachnida	Acarina	Fam.	Morphospecies III	External	18	26	–
Arachnida	Acarina	Fam.	Morphospecies IV	Adapted	–	79	63
Arachnida	Acarina	Fam.	Morphospecies V	Adapted	–	122	–
Arachnida	Araneae	Agelenidae	*Histopona leonardoi* Pantini & Isaia, 2013	External	17	14	–
Arachnida	Araneae	Agelenidae	*Tegenaria silvestris* L. Koch, 1872	Adapted	2	8	4
Arachnida	Araneae	Amaurobiidae	*Amaurobius* sp.	–	2	1	–
Arachnida	Araneae	Dysderidae	*Harpactocrates drassoides* (Simon, 1882)	External	37	34	1
Arachnida	Araneae	Fam.	Immatures indet.	–	2	3	–
Arachnida	Araneae	Gnaphosidae	*Drassodes* sp.	–	–	1	–
Arachnida	Araneae	Linyphiidae	Gen. sp.	Adapted	–	6	2
Arachnida	Araneae	Linyphiidae	*Mansuphantes aridus* (Thorell, 1875)	Adapted	–	1	1
Arachnida	Araneae	Linyphiidae	*Micrargus alpinus* Relys & Weiss, 1997	Adapted	–	1	–
Arachnida	Araneae	Linyphiidae	*Porrhomma convexum* (Westring, 1851)	Adapted	–	2	–
Arachnida	Araneae	Linyphiidae	*Troglohyphantes bornensis* Isaia & Pantini, 2008	Adapted	–	4	15
Arachnida	Araneae	Linyphiidae	*Troglohyphantes lucifer* Isaia, Mammola & Pantini, 2016	Adapted	–	6	1
Arachnida	Araneae	Linyphiidae	*Troglohyphantes* n. sp.	Adapted	2	6	–
Arachnida	Araneae	Lycosidae	*Trochosa hispanica* Simon, 1870	External	1	–	–
Arachnida	Araneae	Nesticidae	*Kryptoesticus eremita* (Simon, 1879)	Adapted	–	3	2
Arachnida	Araneae	Philodromidae	*Philodromus* sp.	External	–	1	–
Arachnida	Araneae	Phrurolithidae	*Phrurolithus festivus* (C. L. Koch, 1835)	External	–	1	–
Arachnida	Araneae	Pimoidae	*Pimoa graphitica* Mammola, Hormiga & Isaia, 2016	Adapted	–	–	2
Arachnida	Araneae	Salticidae	*Saitis barbipes* (Simon, 1868)	External	1	–	–
Arachnida	Araneae	Tetragnathidae	*Meta menardi* (Latreille, 1804)	Adapted	–	1	2
Arachnida	Araneae	Tetragnathidae	*Metellina merianae* (Scopoli 1763)	Adapted	–	1	–
Arachnida	Araneae	Theridiidae	Gen. sp.	–	–	1	–
Arachnida	Araneae	Theridiidae	*Pholcomma gibbum* (Westring, 1851)	External	3	–	–
Arachnida	Opiliones	Dicranolasmatidae	*Dicranolasma soerensenii* Thorell, 1876	External	35	3	–
Arachnida	Opiliones	Ischyropsalidae	*Ischyropsalis dentipalpis* Canestrini, 1872	Adapted	–	–	1
Arachnida	Opiliones	Nemastomatidae	*Nemastoma dentigerum* Canestrini, 1873	External	29	1	–
Arachnida	Opiliones	Nemastomatidae	*Paranemastoma quadripunctatum* (Perty, 1833)	External	19	4	–
Arachnida	Opiliones	Phalangiidae	Gen. sp.	–	1	–	–

Class	Order	Family	Species	Adaptation	Litter	MSS	Cave
Arachnida	Opiliones	Phalangiidae	*Astrobunus bernardinus* Simon, 1879	External	22	1	–
Arachnida	Opiliones	Phalangiidae	*Leiobunum religiosum* (Simon, 1879)	–	–	–	1
Arachnida	Opiliones	Phalangiidae	*Odiellus coronatus* (Roewer, 1911)	External	4	–	–
Arachnida	Opiliones	Trogulidae	*Anelasmocephalus rufitarsis* Simon, 1879	External	17	–	–
Arachnida	Opiliones	Trogulidae	*Trogulus nepaeformis* (Scopoli, 1763)	External	13	1	–
Arachnida	Pseudoscorpiones	Chthoniidae	*Chthonius* (*C.*) *tenuis* L. Koch, 1873	External	10	7	2
Arachnida	Pseudoscorpiones	Chthoniidae	*Chthonius* (*Globochthonius*) *globifer* Simon, 1879	External	6	3	2
Arachnida	Pseudoscorpiones	Chthoniidae	*Chthonius* sp.	–	–	3	–
Arachnida	Pseudoscorpiones	Neobisiidae	*Roncus* sp.	External	11	–	–
Chilopoda	Ord.	Fam.	Gen. sp.	External	161	–	–
Chilopoda	Geophilomorpha	Linotaeniidae	*Strigamia acuminata* (Leach, 1814)	–	–	2	–
Chilopoda	Lithobiomorpha	Lithobiidae	*Eupolybothrus tridentinus* (Fanzago, 1874)	–	–	1	–
Chilopoda	Lithobiomorpha	Lithobiidae	*Lithobius pellicensis* Verhoeff, 1935	–	–	–	3
Chilopoda	Lithobiomorpha	Lithobiidae	*Lithobius pilicornis* Newport, 1844	–	–	1	–
Chilopoda	Scolopendromorpha	Cryptopidae	*Cryptops parisi* Brolemann, 1920	–	–	1	–
Clitellata	Haplotaxida	Lumbricidae	*Lumbricus* sp.	External	12	2	–
Diplopoda	Chordeumatida	Craspedosomatidae	Gen. sp.	External	511	33	4
Diplopoda	Glomerida	Glomeridae	Gen. sp.	External	26	6	
Entognatha	Collembola	Fam.	Morphospecies I	External	45	79	285
Entognatha	Collembola	Fam.	Morphospecies II	Adapted	–	–	323
Entognatha	Collembola	Fam.	Morphospecies III	Adapted	–	321	477
Entognatha	Collembola	Fam.	Morphospecies IV	Adapted	1	127	–
Entognatha	Collembola	Fam.	Morphospecies V	External	22	–	–
Entognatha	Collembola	Fam.	Morphospecies VI	External	75	–	–
Entognatha	Diplura	Fam.	Gen. sp.	External	76	–	–
Gastropoda	Pulmonata	Helicidae	*Helix* sp.	External	35	–	–
Gastropoda	Pulmonata	Limacidae	*Limax* sp.	External	22	36	–
Gastropoda	Pulmonata	Oxychilidae	*Oxychilus* sp.	Adapted	–	22	2
Insecta	Blattodea	Fam.	Larvae indet.	External	–	4	–
Insecta	Blattodea	Blattellidae	*Blattella* sp.	External	10	2	–
Insecta	Coleoptera	Fam.	Larvae indet.	–	18	42	44
Insecta	Coleoptera	Carabidae	*Abax continuus* Ganglbauer, 1891	External	163	3	–
Insecta	Coleoptera	Carabidae	*Bembidion* (*Peryphanes*) *deletum* Audinet Serville, 1821	–	–	1	–
Insecta	Coleoptera	Carabidae	*Binaghites affinis ovalipennis* (Ganglbauer, 1900)	External	1	–	–
Insecta	Coleoptera	Carabidae	*Carabus intricatus* Linne, 1761	External	3	–	–
Insecta	Coleoptera	Carabidae	*Carabus monticola* Dejean, 1826	External	26	–	–
Insecta	Coleoptera	Carabidae	*Cychrus italicus* Bonelli, 1810	External	28	1	–
Insecta	Coleoptera	Carabidae	*Limodromus assimilis* (Paykull, 1790)	External	1	–	–
Insecta	Coleoptera	Carabidae	*Platynus complanatus* Dejean, 1828	External	4	2	1
Insecta	Coleoptera	Carabidae	*Pterostichus* (*Oreophilus*) *externepunctatus* (Dejean, 1828)	External	106	38	–
Insecta	Coleoptera	Carabidae	*Pterostichus* (*Pterostichus*) *rutilans* (Dejean, 1828)	Adapted	–	5	–
Insecta	Coleoptera	Carabidae	*Sphodropsis ghilianii ghilianii* (Schaum, 1858)	Adapted	2	189	381

Class	Order	Family	Species	Adaptation	Litter	MSS	Cave
Insecta	Coleoptera	Carabidae	*Stomis* (*Stomis*) *elegans* Chaudoir, 1861	External	6	–	–
Insecta	Coleoptera	Carabidae	*Trechus modestus* Putzeys, 1874	–	–	1	–
Insecta	Coleoptera	Cholevidae	*Apocatops monguzzii* Giachino & Vailati, 1987	Adapted	–	4	1
Insecta	Coleoptera	Cholevidae	*Bathysciola pumilio* (Reitter, 1884)	Adapted	49	150	34
Insecta	Coleoptera	Cholevidae	*Catops fuliginosus* Erichson, 1837	–	–	1	–
Insecta	Coleoptera	Cholevidae	*Catops subfuscus* Kellner, 1846	Adapted	–	195	175
Insecta	Coleoptera	Cholevidae	*Catops tristis* (Panzer, 1794)	–	1	2	–
Insecta	Coleoptera	Cholevidae	*Catops ventricosus rotundatus* Szymczakowski, 1963	External	161	113	2
Insecta	Coleoptera	Cholevidae	*Choleva angustata* (Fabricius, 1781)	Adapted	–	–	4
Insecta	Coleoptera	Cholevidae	*Dellabeffaella roccae* (Capra, 1924)	Adapted	–	1	991
Insecta	Coleoptera	Cholevidae	*Sciodrepoides watsoni* (Spence, 1815)	Adapted	–	38	–
Insecta	Coleoptera	Colonidae	*Colon* sp.	–	–	4	–
Insecta	Coleoptera	Curculionidae	*Otiorhynchus salicicola* Heyden, 1908	External	11	3	–
Insecta	Coleoptera	Elateridae	Gen. sp.	–	–	1	–
Insecta	Coleoptera	Latridiidae	Gen. sp.	External	4	–	–
Insecta	Coleoptera	Leiodidae	Gen. sp.	–	2	1	–
Insecta	Coleoptera	Ptinidae	Gen. sp.	–	2	1	–
Insecta	Coleoptera	Scaphydidae	Gen. sp.	–	3	–	–
Insecta	Coleoptera	Scarabeidae	Gen. sp.	External	111	–	1
Insecta	Coleoptera	Scydmaenidae	Gen. sp.	External	–	–	1
Insecta	Coleoptera	Silphidae	*Nicrophorus* sp.	External	137	14	–
Insecta	Coleoptera	Silphidae	*Silpha* sp.	External	6	–	–
Insecta	Coleoptera	Staphylinidae	*Bryaxis brachati* Besuchet, 1980	–	–	2	–
Insecta	Coleoptera	Staphylinidae	Gen. sp.	External	111	133	–
Insecta	Coleoptera	Staphylinidae	Gen. sp. (Pselaphinae)	Adapted	–	4	2
Insecta	Dermaptera	Forficulidae	*Forficula* sp.	External	2	2	–
Insecta	Diptera	Fam.	Larvae indet.	–	289	421	787
Insecta	Diptera	Limoniidae	*Chionea* sp.	Adapted	–	37	1
Insecta	Diptera	Limoniidae	Gen. sp.	Adapted	7	570	2085
Insecta	Diptera	Muscidae	Gen. sp.	External	100	79	–
Insecta	Diptera	Phoridae	Gen. sp.	External	1071	1220	802
Insecta	Hymenoptera	Formicidae	Gen. sp.	External	360	678	3
Insecta	Hymenoptera	Vespidae	Gen. sp.	External	4	1	–
Insecta	Orthoptera	Acrididae	Gen. sp.	External	9	–	–
Insecta	Orthoptera	Gryllidae	*Gryllus* sp.	–	2	–	–
Insecta	Orthoptera	Rhaphidophoridae	*Dolichopoda azami septentrionalis* Baccetti & Capra, 1958	Adapted	–	1	14
Insecta	Rhyncota	Fam.	Gen. sp.	–	–	3	–
Insecta	Rhyncota	Pentatomidae	*Pentatoma* sp.	External	7	–	–
Insecta	Rhyncota	Pyrrhocoridae	*Pyrrhocoris apterus* (Linnaeus, 1758)	External	3	–	–
Malaco-straca	Isopoda	Armadillidiidae	Gen. sp.	External	569	14	–
Malaco-straca	Isopoda	Trichoniscidae	*Alpioniscus feneriensis caprae* Parona, 1880	Adapted	–	6	279
Zygentoma	Thysanura	Lepismatidae	*Lepisma* sp.	External	11	1	–

Appendix 2



Graphical abstract.